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16. Exploiting photosystem I as a light-driven reductase

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Photosystem I is one of the two membrane-bound photosystem of plants, algae and cyanobacteria that mediate light-driven electron transport from water to NADPH. Photosystem I generates the most negative redox potential in nature and is extremely efficient in its utilization of light. In plants, the PSI complex consist of at least 19 protein subunits, approximately 175 chlorophyll molecules, 2 phylloquionones and 3 Fe_4S_4 clusters. PSI carries out light-mediated electron transfer to $NADP^+$ using the soluble electron carriers ferredoxin (Fd) and ferredoxin $NADP^+$-oxidoreductase (FNR) to form NADPH. Despite its complex structure and multiple subunits, PSI is a stable protein-pigment complex that can tolerate significant manipulations to many of its peripheral protein subunits without compromising its function and efficiency (for a review see Jensen et al., 2007). PSI is therefore ideal for investigating basic electron transfer reactions and molecular coupling to enzymes like reductases and hydrogenases.
Cytochrome P450s are membrane bound proteins processing an unequalled catalytic versatility, although the most common reactions can be classified as monooxygenations. To complete the catalytic cycle, cytochrome P450 oxidoreductase (CPR) mediates electron transport from NADPH via the one electron carriers FAD and FMN to the heme of membrane bound cytochrome P450 (Wang et al., 1997).

At PSI, FNR interacts with the photoreduced Fd through a FAD cofactor binding domain. Thus, FNR and CPR contain structurally similar FAD domains. Based on these structurally similarities, we want to engineer electron transfer from PSI to P450 and to combine the two membrane complexes, which in nature are localized in the chloroplast and the endoplasmatic reticulum, respectively.
The first step is to connect PSI and the P450 enzyme via the FAD oxidoreductase domain or alternatively via complex bound ferredoxin. Preliminary experiments and results will be presented as will perspectives of formation of this new metabolome that can be used to hydroxylate desired substrates based on a light-driven electron transfer from PSI.

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